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Published in:
Civil and Environmental Engineering Reports

DOI:
10.1515/ceer-2017-0040

Publication date:
2017

Citation for published version (APA):
Krzyzak, J., Pogrzeba, M., Rusinowski, S., Clifton-Brown, J., McCalmont, J., Kiesel, A., Mangold, A., & Mos, M. (2017). Heavy Metal Uptake by Novel Miscanthus Seed-Based Hybrids Cultivated in Heavy Metal Contaminated Soil. *Civil and Environmental Engineering Reports*, *26*(3), 121-132. https://doi.org/10.1515/ceer-2017-0040

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CIVIL AND ENVIRONMENTAL ENGINEERING REPORTS

ISSN 2080-5187

CEER 2017; 26 (3): 121-132

DOI: 10.1515/ceer-2017-0040

Original Research Article

HEAVY METAL UPTAKE BY NOVEL *MISCANTHUS* SEED-BASED HYBRIDS CULTIVATED IN HEAVY METAL CONTAMINATED SOIL

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A b s t r a c t

When heavy metal contaminated soils are excluded from food production, biomass crops offer an alternative commercial opportunity. Perennial crops have potential for phytoremediation. Whilst the conditions at heavy metal contaminated sites are challenging, successful phytoremediation would bring significant economic and social benefits. Seed-based *Miscanthus* hybrids were tested alongside the commercial clone *Miscanthus × giganteus* on arable land, contaminated with Pb, Cd and Zn near Katowice. Before the randomized experimental plots were established ($25m^2$ plots with plant density $2/m^2$) 'time-zero' soil samples were taken to determine initial levels of total (*aqua regia*) and bioavailable ($CaCl_2$ extraction) concentration of Pb, Cd and Zn. After the growing season plant material was sampled during autumn (October, green harvest) and winter (March, brown harvest) to determine differences in heavy metal uptake. Results after the first growing season are presented, including the plot establishment success, biomass yield and heavy metal uptake.

Keywords: *Miscanthus*, seed-based hybrids, heavy metals, phytoremediation

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1. INTRODUCTION

Presence of heavy metals (HMs) in the soil is a common environmental phenomenon, especially in the areas affected by past mining and smelting industries. Such areas are usually highly contaminated due to the dispersion of pollutants (*i.e.* dust emission), and consequently, influence cultivated crops. Therefore, if these contaminated areas are considered for arable land, precautions should be taken and when the limits for HMs content are exceeded, such areas have to be excluded from agricultural production for food and feed purposes [9, 21, 22].

Biomass production using perennial plants for energy purposes may offer an alternative; such an approach could provide multiple benefits in terms of both degraded land management, as well as phytoremediation, due to the stabilization or extraction of toxic elements by plants [15]. There has already been extensive research investigating the potential of different energy crop cultivation in soil contaminated with HMs [1, 11, 12, 19].

Miscanthus × *giganteus* is widely cultured in Europe as a bio-energy crop; it is a sterile, triploid interspecific hybrid produced by the natural cross of *M. sinensis* (diploid) and *M. sacchariflorus* (tetraploid) [5]. This energy grass is characterised by high biomass yields, translocation of nutrients and minerals to the rhizome during senescence, efficient water use and solar energy conversion. However, cultivation of *M.* × *giganteus* in the temperate climates of Europe has a few disadvantages, such as relatively high establishment costs using vegetative propagation methods from the rhizomes and insuffcent hardiness in the first winter following establishment especially at continental locations with harsh winter frost periods [3, 7].

Introducing new germplasm from existing breeding programmes at the University of Aberystwyth can extend the geographical range in which *Miscanthus* can be cultivated and overcome some of the current barriers [8]. Development of techniques to introduce new seed propagated hybrids of *Miscanthus* with the associated novel agronomies and developments in harvesting have been projected to make significant reductions in the cost of producing and harvesting the biomass [3]. Additionaly, little research has been conducted on heavy metal uptake by *Miscanthus* seed-based hybrids as they are currently developed. relatively new on the market. Experiments involving the cultivation of commercial *M.* × *giganteus* in contaminated soils are widely described by many authors [10, 11, 14, 19].

The aim of the current study was to assess the cultivation potential of novel *Miscanthus* seeds-based hybrids on heavy metal contaminated arable land, alongside *M.* × *giganteus* propogated from rhizomes. Tested interspecific hybrids between *M. sinensis* and *M. sacchariflorus* were developed under

Aberystwyth University breeding programme from wild collections across Asia. All material produced under CBD rules with full traceability and agreed profit sharing with donor countries following commercialisation. Hybrids were breeded to be tailored to climate, soils and different end-uses, and currently are at precommercialisation. Establishment success (the ability of tested plants to grow in climate and environment conditions) was presented for all tested hybrids, while biomass production, lead, cadmium and zinc uptake after the first growing season were presented for *Hyb1*, *Hyb2* and *M.* × *giganteus*.

2. MATERIALS AND METHODS

2.1. Site description

The experiment was carried out on contaminated arable land in Bytom (Upper Silesia), Poland (50°20'43.1"N 18°57'17.9"E) on the test site of the Institute for Ecology of Industrial Areas. The soil was contaminated over the last century with zinc, cadmium and lead deposition, resulting from nearby Pb/Zn smelting. Total soil HMs exceed the maximum threshold values described by Polish government regulations [4], excluding this area from food production. Yearly average values of temperature and precipitation sums measured during the first growing season (May 2014 – March 2015) were 10.5°C and 590 mm respectively (Institute of Meteorology and Water Management, Poland).

2.2. Experiment design

Plots were established at the beginning of the 2014 growing season. Four interspecific hybrids of *M. sinensis* and *M. sacchariflorus* (further described as *Hyb1*, *Hyb2, Hyb3 and Hyb4*) were planted and the commercial *M.* × *giganteus* was also established as comparison, each in randomized triplicates.

Each plot (25m^2) was planted with a density of 2 plants per m^2. Seed-based hybrids, provided by Terravesta Ltd., were planted as seedlings germinated and precultivated in the greenhouse, while *M.* × *giganteus* was planted from 7-10 cm long rhizome cuts. Before planting, “time-zero” soil samples were taken from each of the plots to determine basic soil physio-chemical properties, as well as HMs concentrations (both total and bioavailable). No additional fertilizers were used before the plantation establishment, or, during the growing season. At the end of the first growing season and after winter, plant survival rate was determined (by counting the number of plants per plot) to determine plant acclimatisation to soil conditions (autumn) and overwintering rate (spring of the next growing season). Plant samples for HM concentrations were taken at the end of growing season (October, green harvest) and before the next growing season (February, brown harvest). Three randomly selected plant stems from each of the plot were sampled during both harvests. For each of the hybrids nine samples repetition were taken (three stems x three plots of each hybrids). Plant samples were washed with tap and distilled water, chopped and dried in 60°C for 72 hours. After drying, plant material was milled. Moreover, during the brown harvest biomass yield per plant was also determined.

2.3. Soil physico-chemical parameters

Soil physico-chemical parameters were measured on soil sifted through a 2 mm sieve. Soil pH was measured in H_2O (ratio 1:2.5 m/v) with a combination of glass/calomel electrode (OSH 10-10, METRON, Poland) and a pH-meter (CPC-551, Elmetron, Poland) at 20°C. The electrical conductivity was determined by an ESP 2ZM electrode (EUROSENSOR, Poland) according to the Polish norm [17].

Soil texture was evaluated by the hydrometric method according to the Polish norm [18]. Soil organic matter content (OM) was measured by loss on ignition as follows: air dry soil was dried at 105°C for 24 h and then (5 g) heated to 550°C for 4 hours.

2.4. Concentration of heavy metals in soil and plants

The concentrations of the bioavailable metals in the soil were obtained using extraction with 0.01 M $CaCl_2$ [16]. Extraction was conducted with 3 g of air-dried soil (< 2 mm) and 30 mL 0.01 M $CaCl_2$ for 2 hours. Bioavailable metal concentrations (Cd, Pb, Zn) were determined in extracts using a flame atomic absorption spectrometer (iCE 3500 FAAS, Thermo Scientific).

The total concentration of metals in the soil and plant aboveground parts was determined by hot plate digestion and flame atomic absorption spectrometry (SpektrAA 300, Varian INC., USA). Soil samples were digested in aqua regia according to the norm [6], while plant samples were digested in nitric and perchloric acid (4:1 v/v) [20].

2.5. Statistical analysis

One-way ANOVA followed by post-hoc LSD test at $P \leq 0.05$ were used to distinguish differences between different hybrids. To distinguish significant differences in concentration of HMs in shoots between autumn and spring harvest Wilcoxon (at $P \leq 0.05$) and student t (at $P \leq 0.05$) tests were performed for variable without and with normal distribution, respectively.

3. RESULTS AND DISSCUSSION

3.1. Soil characteristics

Physical and chemical soil parameters are presented in Tab. 1. The soil was classified as silty-clay loam. Heavy metal concentrations in the soil exceeded Polish limits for arable soil [4]. The pH was almost neutral, followed by high content of organic matter (OM) and low electrical conductivity (EC). Bioavailable forms of cadmium were high (about 7% of total), whereas the bioavailability of lead was low (close to detection limit).

Table 1. The soil characteristic

Soil parameters	Value
pH	6.47 ± 0.03
EC (µS/cm)	90.63 ± 3.32
Organic matter (%)	5.00 ± 0.11
Texture	silty clay-loam
Total heavy metal concentration (aua regia extraction, mg kg^{-1} d.w.)	
Pb	527 ± 21
Cd	19.9 ± 1.0
Zn	2769 ± 301
$CaCl_2$ extractable metal fraction (mg kg^{-1} d.w.)	
Pb	0.03 ± 0.01
Cd	1.35 ± 0.05
Zn	84.0 ± 5.6

Values are mean ± SE, n=9

3.2. Heavy metal concentration in plant shoots

Lead concentration in aboveground parts of plant is presented in Fig. 1. No statistically significant differences in autumn and spring concentrations were found between tested hybrids and *M.* × *giganteus*. The autumn lead concentration was about seven-fold lower when compared to spring harvest.

Cadmium concentration in aboveground parts of plant is presented in Fig. 2. Statistically significant differences between tested hybrids were found when comparing Cd concentration in plant shoots harvested both during autumn and spring, as well as between autumn and spring harvest within the same hybrid. Cadmium concentrations were higher for all of tested hybrids during winter harvest and the highest values were found for *M.* × *giganteus*, three and two fold higher when compared to *Hyb2* and *Hyb1* respectively.

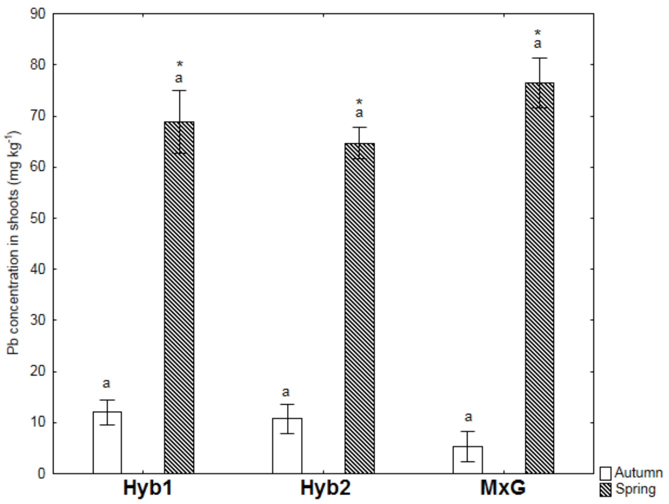

Fig. 1. Lead concentration in plant shoots after autumn and spring harvest. Asterisk (*) denote significantly higher value of concentration, while considering harvest time within one hybrid. Lower case letters (a,b, c) denotes significant differences between different hybrids separately for both harvest times. Values are mean, ±SE, n=9

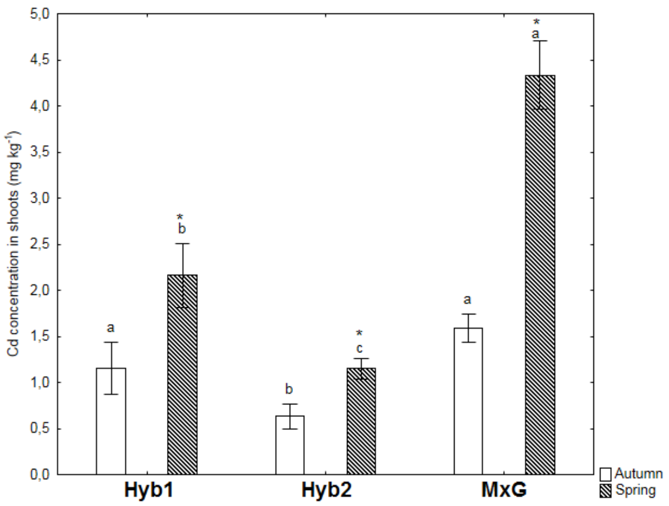

Fig. 2. Cadmium concentration in plant schoots after autumn and spring harvest. Asterisk (*) denote significantly higher value of concentration, while considering harvest time within one hybrid. Lower case letters (a,b, c) denotes significant differences between different hybrids separately for both harvest times. Values are mean, ±SE, n=9

Zinc concentration in aboveground parts of plant is presented in Fig. 3. No statistically significant differences in autumn Zn concentration in plant shoots were found between tested hybrids and *M.* × *giganteus*. Also, no differences were found when comparing autumn and winter samples for *Hyb1* and *Hyb2*, while for *M.* × *giganteus* Zn concentration in shoots sampled in winter harvest were almost two fold higher, when compared to autumn.

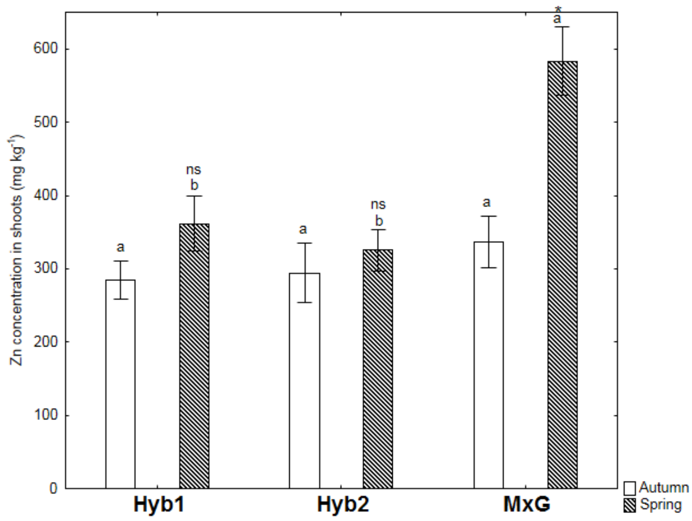

Fig. 3. Zinc concentration in plant shoots after autumn and spring harvest. Asterisk (*) denote significantly higher value of concentration, while considering harvest time within one hybrid. Lower case letters (a,b, c) denotes significant differences between different hybrids separately for both harvest times. Values are mean, ±SE, n=9

Papers concerning the cultivation of *M.* × *giganteus* in contaminated soil are widely available, however, there is a lack of information regarding heavy metal uptake by novel, seeds based hybrids. For example, Pavel et al. [14] tested *M.* × *giganteus* capacity for aided phytostablisation of HM contaminated soil with concentrations of lead and cadmium similar to the presented study. They found that *Miscanthus* in the biomass harvested in spring, accumulated about 25 and 2 mg kg^{-1} of lead and cadmium, respectively. Their results were lower than presented, but reflects to 4th and 5th growing season. Kocoń and Jurga [10] performed experiment, where *M.* × *giganteus* was cultivated in artificially contaminated soil (Pb – 769 mg kg^{-1}, Cd 3.6 mg kg^{-1}, Zn – 1200 mg kg^{-1}). Authors reported, that *M.* × *giganteus* after the first growing season accumulated about 40 and 300 mg kg^{-1} d.w of lead, and cadmium, respectively, which correlates with the results presented here.

Very interesting in this study are the genotypic differences in the uptake and the stability of the HM in the biomass. While *M. x giganteus* seems to store the HM in the aboveground biomass, *Hyb 2* and *1* seem to relocate significant amount of Cd and Zn to the rhizome. For this reason the concentrations of Cd and Zn are increasing far lower over winter in *Hyb 2* and *1*, while for *M. x giganteus* there is a strong increase.

3.3. Biomass productivity

Differences in biomass production of the tested hybrids are presented in Fig.4. The highest biomass production per plant was found for *M.* × *giganteus* and it was almost two fold higher when compared to *Hyb2* and about 50% higher when compared to *Hyb1*. Such big differences might be caused by fact that *M.*× *giganteus* was planted directly from rhizomes and therefore the plants have more available nutrients. Hence, they could possibly grow larger and produce more biomass in the first growing season. The obserevd results are in accordance with the literature [8,13], where after the first growing season the highest yield was measured for *M.* × *giganteus* when compared to seed based hybrids.

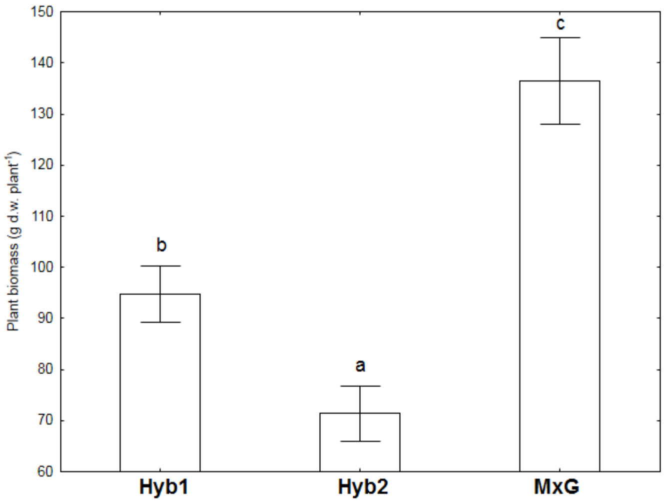

Fig. 4. Plant biomass after winter harvest, expressed in g plant^{-1} d.w.
Lower case letters (a,b, c) denotes significant differences between different hybrids.
Values are mean, ±SE, n=9

3.4. Plants surviving rate

Results of plant survival rate and overwintering sucess are presented in Table 2. At the end of first growing season high plant survival rates were evident, especially for *M.* × *giganteus* as well as *Hyb2*, *Hyb3*, and *Hyb4*, reaching over 90% of survived plants. The lowest survival rate was found for *Hyb1*, but this was still over 80%. The situation changed dramatically when overwintering was assessed at the beginning of the second growing season. A short period of ~10 days of severe frost (from -10 ºC to -16 ºC) together with lack of snow cover in the turn of the years, caused damage to both *Hyb1* and *Hyb2*, but did not severly affect *M.* × *giganteus* and *Hyb3* (96.7 and 97.3% of overwintered plants), while overwintering rate for *Hyb4* was sti*ll 80%*. It could be assumed, that both *Hyb1* and *Hyb2* were not resistant to frost, which implies that they should not be cultivated in the regions where lack of snow cover together with low temperatures can affect plants during winter senescence. Opposite phenomenon was found for *Hyb3* and *Hyb4*, which are well adopted to climatic condition and growing efficiently in the following season.

Table 2. Plant surviving rate after the first growing season

Variants	Autumn	Spring
	Plant surviving (%)	
Hyb1	80.6	0
Hyb2	98.7	0
Hyb3	100	97.3
Hyb4	93.3	80
M. × *giganteus*	99.3	96.7

Mean percentages of surviving plants from replicated plots

It is well known from the literature, that some of the genotypes or varietes of *M. sinensis* and *M. sacchaliflorus* are frost sensitive and might have problems with overwintering after the first growing season [2, 8].

4. CONCLUSIONS

Cultivation of energy crops in heavy metal contaminated soil may provide an opportunity for site restoration. It is crucial to select the correct hybrids which are able both to survive in the contaminated environment as well as climatic conditions. Based on the obtained results it could be concluded, that tested hybrids (*Hyb1* and *Hyb2*) are able to grow in heavy metal contaminated soil, but are sensitive to frost and as such, did not successfully overwinter in the first growth season. However there is high possibility that tested genotypes could survive winter under more favourable conditions. For commercial cultivation of the hybrids selection for frost tolerance are required, as in case of *Hyb3* and *4*, to minimize the risks of plantations failure in continental climates with very low winter temperatures.

5. ADDITIONAL INFORMATION

Research financed by The Polish National Centre for Research and Development (grant agreement No. FACCE SURPLUS/MISCOMAR/01/16) under the flag of Era-Net Cofund FACCE SURPLUS, in the frame of the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI).

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POBIERANIE METALI CIĘŻKICH PRZEZ NASIENNE GENOTYPY MISKANTA UPRAWIANE NA GLEBIE ZANIECZYSZCZONEJ METALAMI CIĘŻKIMI

S t r e s z c z e n i e

Gleby zanieczyszczone metalem ciężkim powinny być wyłączone z produkcji na cele żywnościowe i paszowe, a uprawa roślin energetycznych może stanowić alternatywę pozwalającą na ich wykorzystanie oraz przywrócenie ich ekonomicznej wartości. Rośliny wieloletnie, w tym gatunki roślin energetycznych posiadają potencjał do fitoremediacji gleb zanieczyszczonych. Pomimo tego, że warunki siedliskowe na terenach zanieczyszczonych mogą być trudne, ich wykorzystanie może przynieść znaczne korzyści gospodarcze i społeczne.

W przedstawionej pracy nasienne genotypy miskanta wraz z konwencjonalnym miskantem olbrzymim rozmnażanym z kłączy były uprawiane na glebie rolniczej, zanieczyszczonej metalami ciężkimi. Przed rozpoczęciem eksperymentu pobrano próbki glebowe w celu określenia jej charakterystyki. Po sezonie wegetacyjnym materiał roślinny pobrano do analiz w okresie jesiennym (październik) i zimowym (marzec), aby określić różnice w pobieraniu metali ciężkich pomiędzy testowanymi genotypami jak czasem zbioru. Przedstawiono wyniki po pierwszym sezonie wegetacyjnym, uwzględniając udatność plantacji, produkcję biomasy oraz pobieranie metali ciężkich.

Słowa kluczowe: *Miscanthus*, genotypy nasienne, metale ciężkie, fitoremediacja

Editor received the manuscript: 31.08.2017